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**TITLE OF THE INVENTION (280 characters max)**Anti-A $\beta$ -Antibody**CORRESPONDENCE ADDRESS**

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**ENCLOSED APPLICATION PARTS (check all that apply)**

<input checked="" type="checkbox"/> Specification	Number of pages	18	<input type="checkbox"/> Small Entity Statement
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Date 2/23/04

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51,820

☐ Additional inventors are being named on separately numbered sheets attached hereto**PROVISIONAL APPLICATION FOR PATENT FILING ONLY**

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**ANTI-A $\beta$  ANTIBODY****FIELD OF THE INVENTION**

This invention is in the field of medicine. More particularly, this invention is directed to a composition comprising an anti-A $\beta$  antibody either free of A $\beta$  peptide or with acceptably low amounts thereof.

**BACKGROUND OF THE INVENTION**

A principal component of amyloid plaques is the 39 to 43 amino acid A $\beta$  peptide. This peptide is proteolytically derived from a type I integral membrane protein, the amyloid precursor protein (APP). The predominant forms secreted in cell culture media are A $\beta$  peptide (1-39/40 or X-39/40), whereas the longer forms, A $\beta$  peptide (1-42/43 or X-42/43), which are less soluble and more prone to aggregate, constitute the nucleating seeds for amyloid deposition. Amyloid deposits comprised of A $\beta$  peptide (1-42/43) are associated with conditions and diseases such as Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, vascular dementias, mild cognitive impairment, and the like.

Various therapeutic treatments for conditions and diseases related to abnormal deposits containing the A $\beta$  peptide have focused on preventing A $\beta$  peptide production and/or its aggregation into plaques as well as on reducing or eliminating amyloid plaques. Another treatment approach involves inducing an immunogenic response to A $\beta$  peptide through administration of the peptide by, for example, active immunization (WO 99/27944). However, a recent Phase 2A study utilizing an active immunization approach with synthetic human A $\beta$  1-42 peptide was suspended as soon as four patients experienced clinical signs consistent with inflammation in the central nervous system (CNS). Since the suspension, eleven additional patients developed symptoms associated

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with CNS inflammation. As such, administration of A $\beta$  peptide to treat Alzheimer's disease has caused adverse events and raised safety considerations for the patient.

An alternate immunological means for targeting A $\beta$  peptide is through the administration of antibodies specific for the peptide by, for example, passive immunization. While passive immunization does not establish memory in T and B cells in the manner that active immunization does, the passive approach has not raised the safety concerns that surround active immunization.

Previously, various cell lines, such as K562, M17, HEK 293, CHO, and HUVEC, were shown to produce A $\beta$  peptide (Shoji, *et al.*, *Science*, 258:126-129 (1992); Haass, *et al.*, *Nature*, 359:322-325 (1992)). As such, many cell lines, such as CHO and HEK 293, that can be used to express human antibodies for clinical use endogenously contain APP holoprotein as well as the secretases necessary to cleave APP and, thus, naturally express A $\beta$  peptide.

Surprisingly, during the preparation of anti-A $\beta$  antibodies, it was discovered that A $\beta$  peptide, endogenously produced in most mammalian cell lines commonly used to express antibodies recombinantly, binds to the expressed anti-A $\beta$  antibody and is carried through the cell culture and purification process. Thus, A $\beta$  peptide is found to contaminate recombinantly-produced anti-A $\beta$  antibody material and is now believed to raise potential immunogenicity implications for the patient, making prevention, removal, or reduction of the A $\beta$  peptide of key importance. Furthermore, when the endogenously produced A $\beta$  peptide is non-human, as with a CHO cell line, the immunogenicity implications for non-human A $\beta$  peptide bound to the expressed anti-A $\beta$  antibody may cause even greater concern for patient safety and, thus, make prevention, removal, or reduction of the A $\beta$  peptide vitally important.

## SUMMARY OF THE INVENTION

The present invention provides a composition that is suitable for administration to a human subject comprising an anti-A $\beta$  antibody that is free of A $\beta$  peptide or that has acceptably low levels thereof.

Also, the invention provides a composition that is suitable for administration to a human subject comprising an anti-A $\beta$  antibody that is free of non-human A $\beta$  peptide or that has acceptably low levels thereof.

The invention further provides a composition that is suitable for administration to a human subject comprising an anti-A $\beta$  antibody having an undetectable concentration of A $\beta$  peptide.

One embodiment of the invention provides that the antibody is expressed in NSO cells. Another embodiment provides that the antibody is expressed in cells in which APP production is eliminated through deletion of a specific gene. A further embodiment provides that the antibody is produced in a cell culture that contains a secretase inhibitor. Yet another embodiment provides that the antibody is purified of A $\beta$  peptide by using acidification and size exclusion chromatography.

Additionally, the invention provides for a method of treating human patients with conditions and diseases such as Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, vascular dementias, mild cognitive impairment, and the like using a composition of the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION

For the purposes of the present invention, as disclosed and claimed herein, the following terms are as defined below.

By "antibody" is meant a whole antibody, including without limitation a chimeric, humanized, human, recombinant, transgenic, grafted and single chain antibody, and the like, or any fusion proteins, conjugates, fragments, or derivatives thereof that contain one or more domains that selectively bind A $\beta$  peptide. The expression "anti-A $\beta$  antibody" means an immunoglobulin molecule that recognizes or binds A $\beta$  peptide. The term "A $\beta$  peptide" or "A $\beta$ " in this context includes the 39, 40, 41, 42, and 43 amino acid peptides derived from the amyloid precursor protein (APP) protein *in vivo* by proteolysis, and any fragments of those peptides, such as N-terminally shortened peptides derived from those peptides (e.g., denoted by, for example, x-42, where x = 1, 2, 3, etc.), C-terminally shortened peptides derived from 1-39, 40, 41, and 42 peptides, and peptides shortened at both termini. See SEQ ID NO. 1: Human A $\beta$  peptide and SEQ ID NO. 2: Rodent A $\beta$  peptide (i.e. Mouse, Hamster) for details of each full-length amino acid peptide sequence.

The compositions of the present invention may be made by any of several methods. Details of the following methods are intended to illustrate but not to limit the invention.

In general, recombinant antibody production is accomplished using processes that can be grouped into three major stages: cell line selection, cell culture, and purification. Changes made at any of these stages frequently impact the characteristics of the antibody generated. For the cell line selection stage, a number of variables can affect antibody expression, including the cell line source, vector constructs, and leader sequences. Antibody expression relies upon the use of medium for cell culture. Media modifications including changes to, for example, temperature, nutrients, and dissolved oxygen can impact the product quality. Purification techniques, including, for example, various chromatographic techniques, filtration, and buffer exchange can alter the properties of the desired product, as well as purity and the nature of contaminants. Given these general recombinant antibody production stages, the present invention can be achieved by using particular techniques or making specific modifications at each of these stages.

Methods for making compositions of the present invention involve particular sources of the cell line as well as modifications to the cell line. As previously mentioned, the cell line affects antibody expression. Methods for making compositions of the present invention use mammalian cell lines. Preferably, the mammalian cell line is a hamster, human, or mouse cell line. More preferably, the mammalian cell line is CHO, HEK 293, PER.C6, or NS0. Most preferably, the mammalian cell line is CHO or NS0. Use of NS0 cells to recombinantly produce anti-A $\beta$  antibody is preferable such that the anti-A $\beta$  antibody has an undetectable concentration of A $\beta$  peptide.

Cell lines that lack APP or one of the secretases can be used for generating the recombinant antibodies. Modifications to the cell line can produce this effect. An example of a useful cell line modification involves significantly reducing the amount of A $\beta$  peptide expressed by degrading the RNA transcript for the undesirable protein (e.g. APP or secretase) through a process known as RNA interference. To enable RNA interference, cells can be stably or transiently transfected with a DNA sequence that expresses small hairpin RNA structures which specifically bind to the transcript of interest to initiate cleavage and degradation of that transcript. Alternatively, knock-out mice can be a source of cells lacking the undesirable protein. Cells isolated from these

mice can then be utilized to express the anti-A $\beta$  antibody. Use of these methods reduces or prevents production of A $\beta$  peptide.

Methods for making compositions of the present invention also involve modifications to the cell culture. These methods preferably include incorporating  $\beta$ - or  $\gamma$ -secretase inhibitors in the cell culture to produce anti-A $\beta$  antibody with acceptably low levels of A $\beta$  peptide present. Various  $\beta$ - and  $\gamma$ -secretase inhibitors are known (e.g. U.S. Patent No. 6,486,350, U.S. Patent No. 6,627,739, Dovey *et al.*, *J Neurochem.*, 76:173-181 (2001); Yue-Ming *et al.*, *Nature*, 405: 689-694 (2000)) and can be used for these methods.

Additional methods for making compositions of the present invention increase soluble APP production in the cell culture, thereby reducing in the amount of A $\beta$  peptide produced. For example, soluble APP production is increased with copper addition to CHO cells (Borchardt *et al.*, *Biochem J.*, 344:431-467 (1999)). Copper addition also greatly reduced levels of A $\beta$  peptide in parental CHO-K1 cells and in copper-resistant CHO-CUR3 cells.

Methods for making compositions of the present invention also involve various purification techniques. A preferable method for making compositions of the present invention involves different purification steps including Protein A capture, neutralization, dilution, and acidification of the antibody. After performing these steps, chromatographic techniques capable of separating the antibody from the antigen or from the antibody-antigen complex are used. These chromatographic techniques include but are not limited to size exclusion chromatography.

Additional purification methods for making compositions of the present invention involve agents used to dissociate the anti-A $\beta$  antibody from the A $\beta$  peptide and subsequent separation of the antibody from antigen based on chromatographic differences between these two entities. Preferred dissociative agents include acid, urea, thiocyanate, and detergent. After achieving the dissociation of the antibody and the antigen, chromatographic techniques capable of separating dissociated anti-A $\beta$  antibody from A $\beta$  peptide are used to remove the antigen from the antibody or from the antibody-antigen complex. These chromatographic techniques are preferably size exclusion chromatography, ion-exchange chromatography, reverse phase chromatography, and hydrophobic interaction chromatography. Additionally, tangential flow filtration is used

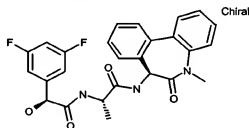


as another technique for separating the antigen from the antibody. Another chromatographic method uses an immobilized antibody to another epitope of A $\beta$  peptide or an antibody with higher affinity to A $\beta$  peptide to isolate and remove the antibody-antigen complex or the antigen.

The compositions of the present invention may be used to treat human patients with conditions and diseases such as Alzheimer's disease, Down's syndrome, cerebral amyloid angiopathy, vascular dementias, mild cognitive impairment, and the like. A $\beta$  peptide may raise potential immunogenicity risks for the patient, with even greater potential health concerns if the A $\beta$  peptide is non-human. As such, prevention, removal or reduction of A $\beta$  peptide is key.

The following examples are intended to illustrate but not limit the invention.

#### Example 1



A  $\gamma$ -secretase inhibitor,

(WO 98/28268), is

added to the HEK 293 cell culture in which an anti-A $\beta$  antibody is being expressed in order to reduce the amount of A $\beta$  peptide naturally expressed by cells. Cell culture samples for this example include a control culture with no inhibitor added, a culture in which 1 nM inhibitor is added at time = 0 and every 24 hours thereafter for 5 days (for a 5 day transfection), and a culture in which 1 nM inhibitor is added at time = 0. These samples are purified using a Protein A column as well as size exclusion chromatography.

The following protocol describes a technique for formic acid denaturation of samples and subsequent electrophoresis through an acid-urea polyacrylamide matrix.

**Day 1****Apparatus setup**

- 1) Clean and assemble Hoeffer plates into a casting stand (16 cm x 14 cm plates with 1.5 mm spacers). Clean plates thoroughly with detergent, rinse in distilled H<sub>2</sub>O (dH<sub>2</sub>O), rinse with acetone, and finally rinse with 100% EtOH.
- 2) Mark plates at 10 cm (22% separating gel) and 11.75 cm (10% step gel).

**Making the Gel and Gel Concentrations**

- 1) Acrylamide (pre-made 40% solution from Bio-Rad/cat#161-0148)
- 2) Gel components:

	<u>4% stacker</u>	<u>10%</u>	<u>22%</u>
Urea	5.76 g	2.88 g	11.56 g
40% Acrylamide	1.6 mL	2 mL	17.6 mL
GAA	1.6 mL	800 $\mu$ L	3.2 mL
TEMED (2.5%)	450 $\mu$ L	200 $\mu$ L	600 $\mu$ L
APS(10%)	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
dH <sub>2</sub> O	to 16 mL	to 8 mL	to 32 mL

Add urea, acrylamide, glacial acetic acid (GAA), and dH<sub>2</sub>O into 50 mL conical tubes. Vortex briefly and incubate in a 55 °C water bath. Vortex every few minutes until the urea is completely in solution. Degas 22% and 10% solutions and place solutions on ice, leaving the 4% stacker in the water bath.

**Pouring the Gel (at room temperature)**

- 1) Add N,N,N',N'-tetramethylethylenediamine (TEMED) to the 22% solution and gently invert several times to mix. Next add the 10% APS (ammonium persulfate) and invert several times. Using a 25 mL pipette, slowly pour solution between the plates until it reaches the 10 cm mark.
- 2) Carefully add a 750  $\mu$ L overlay of 5% GAA. Make sure to use a P1000 pipette to very slowly let the 5% GAA run down one side of the glass plates onto the 22% resolving gel.
- 3) Allow to polymerize at room temperature for at least 1 hour.

- 4) Add the TEMED and 10% APS to the 10%-acrylamide solution in the same manner as for the 22%. Remove the overlay and add the solution until reaching the second mark at 11.75 cm. Again, carefully pour a 750  $\mu$ L overlay of 5% GAA as above. Allow the gel to polymerize for 30 minutes.
- 5) Prior to adding the 4% stacker solution, place the gel stand in a 37 °C incubator for 15 minutes to warm gels. This aids in the wells polymerizing correctly. Degas the 4% stacker solution and keep at 55 °C. Remove the overlay. Add the TEMED, invert several times, and then add the 10% APS. Quickly pour this solution and insert a clean-dry 12-well comb (or a 15-well comb). After pouring the stacker, place the stand back in the incubator for 15 minutes then remove. Allow to polymerize on the benchtop. The stacker will take at least 1.5 hours to polymerize completely.

\*Note: During the polymerization steps, air pockets may appear in the resolving gel. These pockets form due to the change in temperature of the gel during and after polymerization. These spaces do not affect the performance of the technique.

#### Sample Preparation

Analysis upwards of 3 mg of protein in a single lane will achieve reasonable band results. Conditions for analyzing protein are as follows.

##### Sample:

30  $\mu$ L of 100 mg/mL protein (maximum concentration of protein)

80  $\mu$ L formic acid (98%) (ICN cat#15162-90)

20  $\mu$ L Acid Loading Buffer (80% formic acid, 60% sucrose and 0.02% methyl green);

Make buffer by dissolving 6 g sucrose in ~8 mL of ~99% formic acid. Heat and agitate the mixture. After the sucrose has dissolved, Q.S. the solution to 10 mL with ~99% formic acid. Add 2  $\mu$ L of 1% methyl green solution.)

1  $\mu$ L  $\beta$ -Mercaptoethanol

\*Note: Adjust volumes as needed. However, ensure that the final formic acid concentration is always between 70% to 90%.

**Ladders:**

Pharmacia molecular weight markers, M.W. Range 2,512-16,949 (cat#80-1129-83).

Reconstitute protein in 1 mL PBS. Freeze 10  $\mu$ L aliquots at  $-20^{\circ}\text{C}$ . Thaw 1 aliquot for every ladder needed and add 90  $\mu$ L of formic acid (98%), 20  $\mu$ L of Acid Loading Buffer, and 1  $\mu$ L of  $\beta$ -Mercaptoethanol.

**\*Note:** Do not reconstitute these ladders according to manufacturer's instructions (because SDS will cause the ladder to smear).

**BSA blanks:**

Distortion occurs for the outside two lanes of every gel run. To help minimize the negative effects that this distortion creates, load BSA blanks in the outside lanes on either side of the gel. BSA blanks = 1  $\mu$ L of 5% BSA, 90  $\mu$ L formic acid (98%), 20  $\mu$ L Acid Loading Buffer, and 1  $\mu$ L of  $\beta$ - Mercaptoethanol.

**Running the Gel**

**Pre-Run:** Assemble the apparatus in a cold room. Fill the bottom chamber (three-fourths the volume) with pre-chilled Acid Gel Running Buffer (~3 liters). Add 1 liter of buffer to the top reservoir. Pre-run the gel Anode to Cathode at 250 volts for 30 minutes.

**\*Remove** any air bubbles at the bottom of the gel and verify that the buffer has not leaked from the top reservoir.

**\*\*Acid Gel Running Buffer** = 250 mL glacial acetic acid + 3750 mL dH<sub>2</sub>O

**Load Samples:** Rinse wells with buffer prior to loading samples to help remove excess urea. Load samples, ladders, and outside BSA blanks.

**Step-Voltage:** Run the gel Anode to Cathode as follows:

- 15 minutes at 25 volts
- 15 minutes at 50 volts
- 15 minutes at 100 volts
- 15 minutes at 200 volts
- ~ 15 hours at 275 volts

**\*\*Run** the gel overnight until the dye front is ~ 2 to 2.5cm from the bottom, which is generally the next morning if the gel was started in the late afternoon.

**Day 2****Transfer Conditions for the Acid Urea Gel (run in 4°C cold room)**

The night before transfer, make the following buffer and store in the cold room.

**Transfer Buffer:**

Tris-Base	12.36 g
Glycine	57.6 g
Methanol	800 mL
dH <sub>2</sub> O	Up to 4 liters.

Neutralize the acid-urea gel prior to transfer. Carefully remove the gel and place it in a washed glass tray. Add 200 mL of transfer buffer and rock gently for 15 minutes. Repeat this wash step a total of four times. Perform transfer as per normal (2.5 hours at 100 volts).

**Ponceau S Staining and Destaining**

After transfer, visualize the proteins in the ladder by Ponceau-S staining. The nitrocellulose is stained for 5 minutes in a solution of 0.1% Pon-S in 5% acetic acid. After destaining the membrane with dH<sub>2</sub>O (three very short washes), digitally scan the membrane and mark the ladders with a dull pencil. Save the file.

\*Note: This ladder is used as solely for alignment purposes. This technique separates peptides according to their molecular weight (size) and charge. For example, two peptides of the same molecular weight but with differing charges probably do not have the same net mobility. Because of these issues, always run A $\beta$  peptide standards in at least one well, which enables accurate A $\beta$  peptide identification.

\*\*Important: All bands from the peptide standard ladder do not transfer (see figure below).

**Western Blot Conditions**

Boil nitrocellulose membrane in PBS for 5 minutes. Proceed with usual Western conditions.

Blocking Step:

Block membrane in 5% Milk in 1X tris-buffered saline/0.125% Tween 20® (TBS/T) for 45 minutes at 50 mL volume.

Primary Antibody

Use a selected number of anti-A $\beta$  antibodies (e.g. 3) such that the binding epitopes of the selected antibodies bind to different regions on the A $\beta$  peptide. Ensure that the selected antibodies also allow for standard visualization of at least 79 pg. Primary antibody solution is in 0.5% Milk TBS/T with, for example, 1:1000 dilution of the selected antibodies at 20 mL total volume. Leave in primary antibody overnight on a rocker.

**Day 3**

Wash Membrane

Wash the membrane 3X in 1% BSA in TBS/T for 10-15 minutes each.

Secondary Antibody

Secondary antibody solution is in 1% Milk TBS/T with 1:6000 dilution of anti-mouse HRP (Catalog# 7076 from Cell Signaling) at 50 mL total volume. Leave membrane in secondary antibody for 3 hours.

After secondary antibody, wash the membrane 3X with TBS/T for 15 minutes each.

Development

Place membrane in enhanced chemiluminescence (ECL) solution (Pierce Super Signal West Pico Catalog # 34080) for 5 minutes prior to development.

Coomassie Blue staining, Ponceau-S staining, and A $\beta$  Western Blot Example

A



B



C



- A) Individual samples of A $\beta$ 40 and A $\beta$ 42 (9  $\mu$ g each) are run along side a peptide standard ladder. At completion of the run, the gel is stained by Coomassie Blue (order: A $\beta$ 42, A $\beta$ 40, and ladder).
- B) Plasma samples (30  $\mu$ L) from a non-transgenic mouse are spiked with exogenous A $\beta$ 40 and A $\beta$ 42 (5 ng each) and one lane containing both. The Ponceau-S stained membrane is shown. Prior to transfer, one lane containing the peptide ladder is cut and stained with Coomassie Blue. When the Coomassie Blue ladder is aligned with the Ponceau-S stained nitrocellulose, it is readily apparent that the 10.7 kDa and 6.2 kDa bands do not transfer.
- C) For the Western blot of the nitrocellulose membrane for A $\beta$  peptide, this autorad is aligned with the Coomassie Blue stained ladder for size comparison. The bands correspond well to the results seen for the Coomassie Blue stained A $\beta$ 40 and A $\beta$ 42.

The maximum sensitivity of this procedure is dependent upon the reagents used during the Western blotting procedure. For the example detailed above, the maximum sensitivity of this assay is ~1 pg/μg IgG. For the samples, the IgG concentration (mg/mL) is determined by measuring the absorbance at 280 nm and dividing that value by an extinction coefficient of 1.4. Analyses for samples generated from this example provide results as follows:

Table 1. Acid urea gel analysis of purified antibody from cells either with or without a  $\gamma$ -secretase inhibitor.

Samples	FL <sup>1</sup> (pg/μg IgG)	T1 <sup>2</sup> (pg/μg IgG)	T2 <sup>3</sup> (pg/μg IgG)	Total hAβ40 (pg/μg IgG)
No inhibitor	70	38	70	178
Inhibitor every 24 hours for 5 days	4	0	3	7
Inhibitor at T=0	56	27	52	135

<sup>1</sup>Full length hAβ40

<sup>2</sup>N-terminal truncated hAβ40, #1

<sup>3</sup>N-terminal truncated hAβ40, #2

### Example 2

An anti-Aβ antibody is expressed from HEK 293 cells grown in cell culture. The antibody is purified by applying the culture medium to a Protein A column and is eluted with 100 mM glycine buffer, pH 3.1. The Protein A pool is adjusted to about pH 7.4 by adding Tris buffer. This pool is then adjusted to about pH 2 by diluting 1:1 into 1 M glycine, pH2. After about 10 minutes incubation at room temperature, the acidified pool is subjected to size exclusion chromatography on a 60 cm long Superdex 200 column (Amersham) using a mobile phase of 50 mM glycine, 150 mM NaCl, pH 2 at a flow rate of 30 cm/hr. The antibody eluted from the size exclusion column is neutralized by adding Tris buffer and is dialyzed against PBS at pH 7.4. Denaturing acid/urea gradient polyacrylamide gel analyses (see Example 1 for further description of this technique)



provide mass estimations of A $\beta$  peptide in units of pg per  $\mu$ g IgG for samples generated from this example as follows:

Table 2. Acid urea gel analysis of purified antibody from HEK 293 cells: size exclusion chromatography only (no acidification) or acidification and size exclusion chromatography.

Sample	FL <sup>1</sup> (pg/ $\mu$ g IgG)	T1 <sup>2</sup> (pg/ $\mu$ g IgG)	T2 <sup>3</sup> (pg/ $\mu$ g IgG)	Total hA $\beta$ 40 (pg/ $\mu$ g IgG)
HEK 293, No Acid, SEC	39	0	0	39
HEK 293, Acid and SEC	11	0	0	11

<sup>1</sup>Full length hA $\beta$ 40

<sup>2</sup>N-terminal truncated hA $\beta$ 40, #1

<sup>3</sup>N-terminal truncated hA $\beta$ 40, #2

### Example 3

An anti-A $\beta$  antibody is expressed from NS0 cells grown in cell culture. The antibody is purified by applying the culture medium to a Protein A column and is eluted with 100 mM glycine buffer, pH 3.1. The Protein A pool is adjusted to about pH 7.4 by adding Tris buffer. This pool is then diluted 1:1 with PBS and is subjected to size exclusion chromatography on a 60 cm long Superdex 200 column (Amersham) using a mobile phase of PBS, 150 mM NaCl, pH 7.4 at a flow rate of 30 cm/hr. The antibody eluted from the size exclusion column is dialyzed against PBS at pH 7.4. No A $\beta$  peptide is detected using denaturing acid/urea gradient polyacrylamide gel analysis.

ELISA analysis is used to quantitate the concentration of A $\beta$  peptide. Wells of a 96-well ELISA plate (Nunc MaxiSorp™ F96 or C96) are coated with anti-A $\beta$  antibodies (e.g. 2 or more) – that will recognize epitopes outside of the central A $\beta$  peptide (e.g. 17-25) binding region – at a concentration of, for example, 7.5  $\mu$ g/mL of each antibody in a coating buffer overnight at refrigerated conditions. After aspirating the coating solution from the plates, wells are blocked with 300  $\mu$ L/well of HBST/Blotto (0.25% w/v nonfat dry milk in HEPES-Buffered Saline (10 mM and 150 mM, respectively) with EDTA

(3mM) and Tween 20® (0.5% w/v)) for 1 to 2 hours at room temperature and then are washed 1X with Washing Buffer (1X PBS with 0.1% v/v Tween 20®) and are aspirated. Samples containing the antibody are appropriately diluted in HBST/Blotto and are applied to ELISA plates (100 µL per well). Equivalent dilutions are spiked with an additional 0.4 ng/mL synthetic rodent Aβ peptide 1-40 to ensure accurate quantitation in the diluted sample matrix. As a standard, synthetic rodent Aβ peptide 1-40 along with purified anti-Aβ antibody (with < 1 ppm total rodent Aβ peptide) is used. Control samples containing antibody (for total Aβ peptide control) and synthetic rodent Aβ peptide 1-42 spiked into the antibody (for Aβ peptide 1-42 control) are also tested. The ELISA plate is incubated for 1 to 2 hours at room temperature. The wells are washed 4X with Washing Buffer and are aspirated. Then, 100 µL/well of diluted (1:10,000) donkey anti-human IgG – alkaline phosphatase conjugate (Jackson ImmunoResearch, # 709-056-149) in HBST/Blotto is applied to each well. After incubating for 1 to 2 hours at room temperature, washing 4X with Washing Buffer, and aspirating the wells, 100 µl of 1.0 mg/mL pNPP substrate (Kirkegaard & Perry Laboratories, Inc., # 50-80-00) solution in 1X DEA buffer solution is added to each well. The ELISA plate is incubated at room temperature with periodic shaking. Absorbance is read at 405 nm using a microplate reader when the color develops sufficiently (usually 2.0 to 2.5 absorbance units) for the standards. The limit of detection is 0.02 ng/mL. The limit of quantitation is 0.1 ng/mL. Concentration determinations for the standards are determined by AAA analysis. For the samples, the IgG concentration (mg/mL) is determined by measuring the absorbance at 280 nm and dividing that value by an extinction coefficient of 1.4. No Aβ peptide is detected using ELISA analysis.

We claim:

1. A composition that is suitable for administration to a human subject comprising an anti-A $\beta$  antibody that is free of A $\beta$  peptide or that has acceptably low levels thereof.
2. The composition of Claim 1 wherein A $\beta$  peptide is non-human A $\beta$  peptide.
3. A composition that is suitable for administration to a human subject comprising an anti-A $\beta$  antibody having an undetectable concentration of A $\beta$  peptide.

SEQ ID NO. 1: Human A $\beta$  peptide

Asp	Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln
1				5					10					15
Lys	Leu	Val	Phe	Phe	Ala	Glu	Asp	Val	Gly	Ser	Asn	Lys	Gly	Ala
				20					25					30
Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala	Thr		
				35					40					

SEQ ID NO. 2: Rodent A $\beta$  peptide (i.e. Mouse, Hamster)

Asp	Ala	Glu	Phe	Gly	His	Asp	Ser	Gly	Phe	Glu	Val	Arg	His	Gln
1				5					10					15
Lys	Leu	Val	Phe	Phe	Ala	Glu	Asp	Val	Gly	Ser	Asn	Lys	Gly	Ala
				20					25					30
Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala	Thr		
				35					40					

ABSTRACT

This present invention provides a composition that is suitable for administration to a human subject comprising an anti-A $\beta$  antibody that is free of A $\beta$  peptide or that has acceptably low levels thereof, free of non-human A $\beta$  peptide or that has acceptably low levels thereof, or having an undetectable concentration of A $\beta$  peptide.